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Retention and Selectivity of the Nalidixic Acid and Enoxacin by Hydrophilic Interaction Liquid Chromatography on Various Polar Columns with Different Chain Lengths

Bashaer A. Al-Phalahy[1,*], Raad Radi Karabat [2], Ali Amer Waheb[3], Mustafa J. Mohammed[4], Ashraf Saad Rasheed[5]

[1] Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq
[2] Middle Technical University, Institute of Medical Technology Al_mansour, Baghdad, Iraq.
[3] Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq.
[4] Department of Chemistry, College of Education, AL-Iraqia University, Baghdad, Iraq.
[5] Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.

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Abstract

This research aims to provide a comprehensive view of the separation mechanisms of HILIC stationary phases in various applications. This study was conducted to understand the retention behavior of nalidixic acid and enoxacin, to show the effect of alkyl chain length on their selectivity, and to develop a simple and sensitive HILIC method for their determination in pharmaceutical formulations. Two lab-made columns (ZIC-HILIC-1 and ZIC-HILIC-3) were used to examine the retention behavior of nalidixic acid and Enoxacin. The retention mechanism in this research was studied by investigating the effect of parameters that affect the selectivity of the separation, which include (acetonitrile content, pH, and buffer concentration). The zwitterionic columns are characterized by their different alkyl spacer chain length, which have been employed to examine the retention behavior of select drugs. The eluent consists of acetonitrile: 10mM acetate buffer (90:10 v/v, pH 4.75) equipped with a UV detector set at 220nm, at a flow rate of 0.75 mL/min. This research developed a ZIC-HILIC method to determine nalidixic acid and enoxacin in pharmaceutical formulations. The technique exhibited linearity in the concentration range (0.1– 6 µg/mL) and 0.1-12 µg/mL for nalidixic acid and Enoxacin, respectively. LOD is 0.070 and 0.04 µg/mL for nalidixic acid, and LOD is 0.080 and 0.060 µg/mL for enoxacin in ZIC-HILIC-1 and ZIC-HILIC-3 columns, respectively.

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*Corresponding Author: email: bashaerabbas113@gmail.com

1. Introduction

Infectious diseases were the predominant factor of human illness, leading to mortality and bacterial infections, representing approximately one-third of these cases[1]. The discovery of antibiotic agents created new pathways for treating bacterial infections[2]. Here, we pay particular attention to a vital class of synthetic antibiotics, the quinolones. They constitute one of the most potent classes of antibiotics and have evolved to become highly effective in treating infectious diseases[3]. Quinolones hasten bacterial cell death by inhibiting DNA synthesis and facilitating DNA breakage within the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase[4-6]. Nalidixic acid (NAL) is a basic member of synthetic quinolone antibiotics and is a bacterial DNA gyrase inhibitor [7, 8]. Enoxacin (ENX) is an oral administered fluoroquinolone antibacterial agent with broad-spectrum activity[9]. ENX has been employed in clinical practice to treat genitourinary tract infections [10]. Various chromatographic methods

for the separation and determination of NAL and ENX (Figure 1) in pharmaceutical and human samples[11, 12] and plant and soil samples[13].

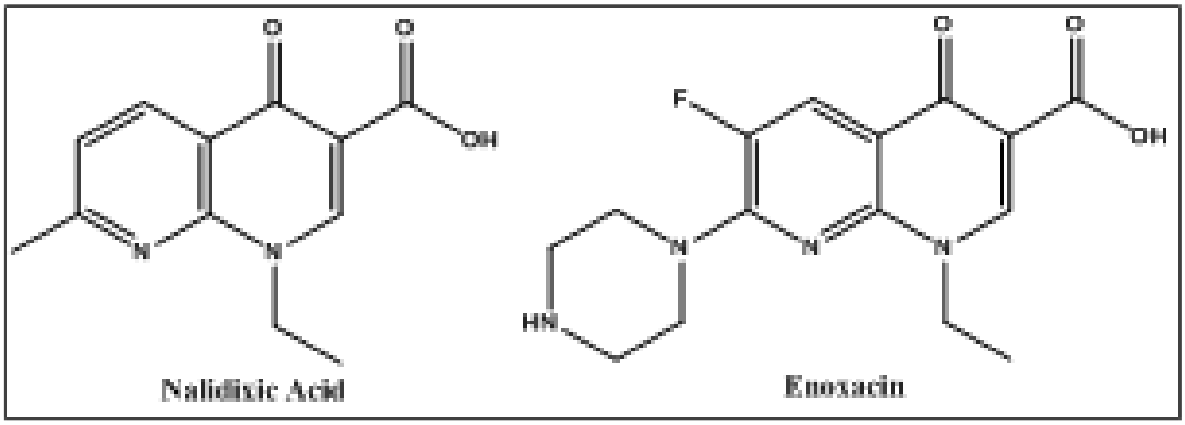

Figure 1: Chemical Structures of Nalidixic Acid (NAL) and Enoxacin (ENX)

Although the main separation technique used in contemporary chromatographic science is reversed-phase liquid chromatography (RPLC), it is not necessarily ideal for separating polar compounds because of poor retention, hence lowering resolution[14-17]. Hydrophilic interaction liquid chromatography (HILIC) has proven to be an effective and significant technique for the analysis of polar and hydrophilic substances[18, 19] utilizing polar and hydrophilic stationary phases[20-22] with eluent consisting of water and organic solvent such as acetonitrile (ACN)[23]. The primary separation mechanism in HILIC involves solutes partitioning between the water layer partially immobilized on the HILIC stationary phase and the mobile phase rich in ACN [24]. Other crucial interactions, such as electrostatic interactions[25, 26] and hydrogen[27, 28] bonding, play significant roles in retaining hydrophilic compounds in HILIC. The first aim of this research is to gain insights into the retention behavior of NAL and ENX in HILIC and to investigate various separation mechanisms. In our previous works[29, 30], we investigated the effect of the alkyl chain length between polar functional groups of the column on the retention of various analytes. We observed that an increase in spacer chain length led to an increase in the retention factor of our target analytes. To our knowledge, no published research has examined this impact on the behavior of NAL and ENX. Therefore, the second objective of this study is to explore this influence. The third aim of this work develop a simple, accurate, and sensitive HILIC technique for the evaluation of NAL and ENX in pharmaceutical formulations. In comparison with previous work, the retention mechanism of the analyte was determined by a single mechanism, such as partition, electrostatic interaction, or the stationary phase. In our study, the retention behavior of NAL and ENX is influenced by hydrophilic partition, electrostatic effect, stationary phase structure, and mobile phase composition.

2. Experimental section

2.1. Chemicals

Nalidixic acid (≥98%) and Enoxacin sesquihydrate (98%) were obtained from Sigma-Aldrich. ACN (≥99%) was acquired from Fisher Scientific. Merck provided 99.8% acetic acid and 99.99% sodium acetate. The water was purified using a Milli-Q system (Millipore, USA) and used in all tests. Nalidixic acid tablets were obtained from Glitz Pharma (500 mg, Nalid, Pakistan, Sample 1), Sun Pharmaceutical Industries Ltd. (500 mg, NALIDIXIC, India, Sample 2), and Sakhiya Pharma Chem (125 mg, Nalidixic Acid, India, Sample 3), respectively. Enoxacin tablets were obtained from Abbott Laboratories Ltd. (400 mg, Enoxabid, Pakistan, Sample 1), ZAFA Pharmaceutical Laboratories (400 mg, Enoxazan, Pakistan, Sample 2), and Shandong Luoxin Pharmaceutical Group HENGXIN Pharmaceutical Co., Ltd. (200 mg, Enoxacin, Pakistan, Sample 3), respectively.

2.2. HPLC instrument

Using an HPLC system built by Merck-Hitachi, chromatographic analysis was carried out with a UV detector of type L-4200. The named firms provided

the L-6200 pump and in-line degasser. Furthermore, included in the system was a 20 µL injection loop. The chromatographic data were handled on the N2000 workstation.

2.3. Stationary phases and chromatographic conditions

The selection of an appropriate stationary phase is essential for conducting effective analyses. This study selected two lab-made columns (ZIC-HILIC-1 and ZIC-HILIC-3, 100 mm × 4.6 mm I.D.) with different chain lengths. The optimal mobile phase is ACN:10mM acetate buffer (90:10 v/v, pH 4.75) to analyze the desired quinolones. The injection volume was set at 20 µL, and elution was performed at a flow rate of 0.75 mL/min at 25°C. The ultraviolet area at a wavelength of 220 nm was used to analyze NAL and ENO.

2.4. Preparation of standard solutions

Standard solutions of NAL and ENX were prepared daily. To avoid the solutions becoming photo-degraded, they were always kept in bottles of opaque glass and in a cool ambient (6 °C). The solutions were prepared by dissolving 5 mg of NAL and ENO in 50 mL of eluent, and a stock solution of the selected quinolone drugs was prepared (100 µg/ ml).

2.5. Preparation of the pharmaceutical samples

Twelve tablets of NAL and ENX were weighed, and the combined crushed and powdered contents equivalent to one tablet were transferred into a 100 mL flask. This mixture was then diluted with mobile phase ACN: H_2O(90:10 v/v) and subjected to ultrasonication for approximately 15 min. The volume was then adjusted to the mark with the eluent. Filtration was carried out using a syringe filter (0.45 µm) before injecting into liquid chromatography.

3. Results and Discussion

Throughout the investigation, two lab-made stationary phases characterized by inner quaternary amines and outer sulfonate group (ZIC-HILIC-1 and ZIC-HILIC-3) were examined. These two stationary phases contain one and three methylene groups between charged function groups. This difference in the structure of these columns will be investigated to separate these drugs. The impact of ACN content, pH, and ionic strength on the retention behaviour of NAL and ENX in the eluents under investigation was evaluated to explore the separation mechanism.

3.1. Effect of ACN concentration on retention behavior of NAL and ENX

The concentration of organic solvents within the mobile phase significantly influences retention and selectivity in HILIC [23]. ACN is the predominant organic modifier in the HILIC mode[31]. The impact of varying ACN content on the retention was examined in the range of 70–90% (v/v keeping the buffer salt concentration constant at 10 mM and adjusting the pH of the aqueous phase to 4.75. Figure 2 illustrates the changes in the retention factor for NAL and ENX concerning the ACN content in the mobile phase. Because of the increase in the amount of acetonitrile from 70% to 90%, there was a modest increase in the amount of retained enoxacin. In contrast, the retention of nalidixic acid decreased in proportion to the same increase in the quantity of acetonitrile. The hydrophilic interaction (HILIC) found in enoxacin is exhibited. On the other hand, Nalidixic acid demonstrates hydrophobic (RP) properties even when the concentration of ACN eluent is increased from 70% to 90% (v/v). The reason for the difference in behavior is attributed to the values of the octanol-water distribution coefficient (logP) for the two drugs (NAL and ENX), as the logP for NAL is 0.87 and for ENX is -1.028.

3.2. Effect of buffer concentration on retention behavior of NAL and ENX

Salts enhance the retention of solutes in HILIC by increasing the volume of the water layer immobilized on the stationary phase[30]. Early investigations suggested that the eluent's absence in salts caused significantly prolonged retention times and broad peaks [32]. Sodium acetate was selected in this study due to its high solubility in an eluent containing significant levels of ACN. The retention behaviour of NAL and ENX was examined in the range of 10–50 mM under the influence of buffer salt concentration. Sodium acetate was employed at pH 4.75 for the ZIC-HILIC-1 and ZIC-HILIC-3 columns; CAN/buffer 90/10 (v/v) made up eluent.

The retention of NAL and ENX decreases as the buffer concentration increases during the process. Based on the information shown in Figure 3, this slope was determined by employing the standard ion exchange columns REF. The slopes obtained from these columns were identical to (0.053, 0.061) and (0.042, 0.047) for NAL and ENX. The pKa value for NAL is 3.55, and the pKa value for ENX is 4.24. Additionally, the isoelectric points for NAL and ENX are 7.05 and 6.79, respectively. Cationic forms will be exhibited by both the NAL and the ENX as a consequence. After that, the cation exchange

provided the basis for the interaction between the NAL and ENX columns and the HILIC columns.

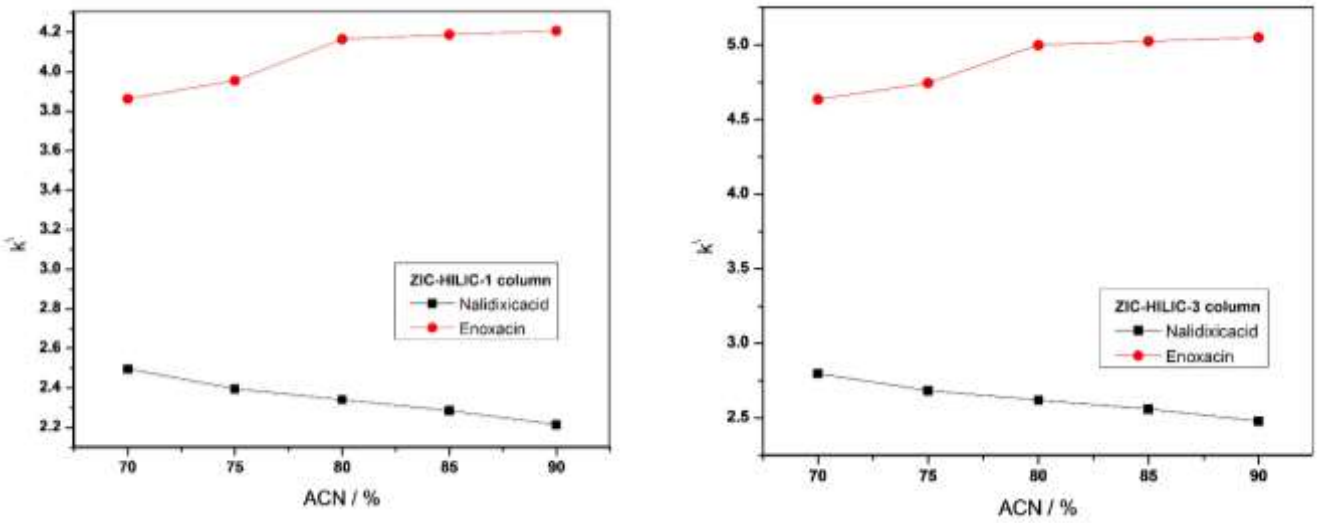

Figure 2: Influence of acetonitrile ratio of the eluent on the retention behavior of nalidixic acid and enoxacin utilizing separation columns.

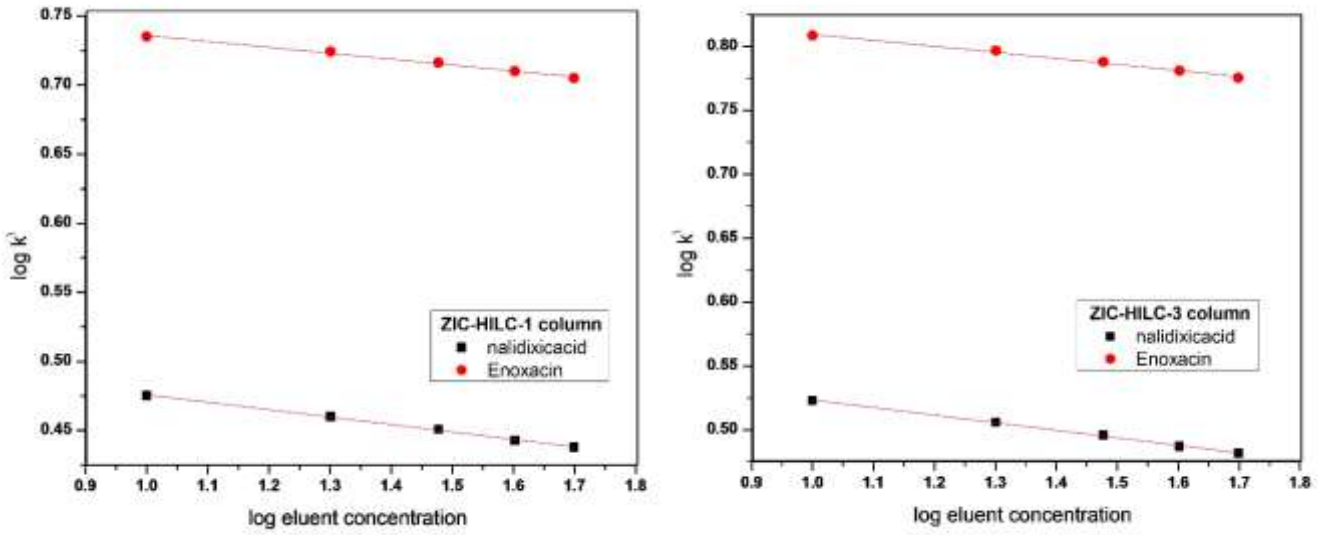

Figure 3. Influence of buffer concentration of the eluent on the retention behavior of nalidixic acid and enoxacin utilizing separation columns.

3.3. Effect of mobile phase pH on retention behavior of NAL and ENX

The pH of the eluent changes the electrostatic interactions between the charged functional groups of the stationary phase and the charged solutes[33]. Separating selectivity is more influenced by the pH fluctuation in the eluent than by variation in organic solvent composition[34]. In this part of the study, a pH range of 3.5–5.5 was used with an acetate buffer at a concentration of 10 and 90% ACN ratio. Because the amino group in NAL and ENX deprotonates, increasing the pH of the buffer reduces the retention of the NAL and ENX as shown in Figure 4.

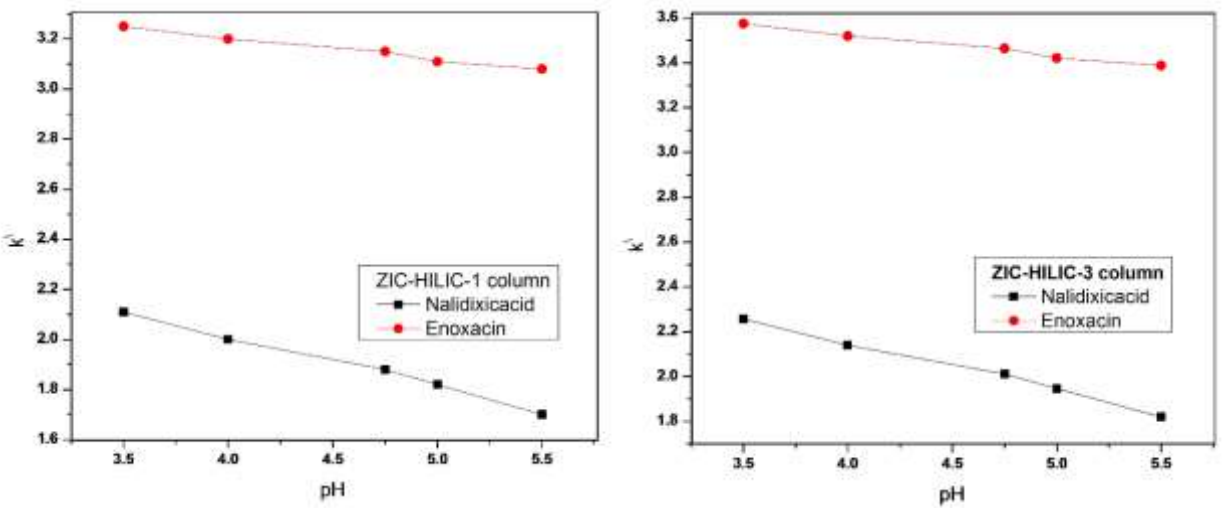

Figure 4. Influence of pH of the eluent on the retention behavior of nalidixic acid and enoxacin utilizing separation columns.

3.4. Optimizing Separation of NAL and ENX

To achieve high resolution within a short duration for NAL and ENX, characterized by sharp, symmetric peaks, it was imperative to examine how various factors influence sensitivity, selectivity, and separation efficiency. Two lab-made columns (ZIC-HILIC-1 and ZIC-HILIC-3) were used to examine the separation mechanism for the analyzed components. Various concentrations of the mobile phase components were tested, and the eluent composed of ACN: acetate buffer (90:10) gave the best results. Salt concentration is crucial in HPLC, significantly affecting resolution and retention time. Various pH levels ranging from 3.5 to 5.5 and buffer concentrations from 10 to 50 were examined. The optimal results were achieved at pH 4.75, 10mM. The highest retention for the selected drugs was obtained using ZIC-HILIC-3[35]. The explanation for this behavior is that methylene groups are positioned among charged groups in ZIC stationary phases. The ZIC-HILIC-3 has a longer spacer chain length between charged groups, causing varying flexibility and forming intra- and intermolecular ion pairs. As a result, when the alkyl chain between the charges of the stationary phases increases causes a strong electrostatic interaction and improves the hydrophilic partition, leading to an increase in the retention and selection of the NAL and ENX (Figure 5).

Table 1. Analytical parameters of NAL and ENX analysis employing two different HILIC columns, concentration, LOD, and LOQ values are provided in µg/mL.

Intra-Day Analysis (n=6)			Inter-to-Day Analysis (n=6)	
ZIC-HILIC-1column				
NAL Taken(µg/mL)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
1.00	99.42	0.52	99.63	0.61
2.00	100.08	0.42	100.11	0.40
ENX Taken(µg/mL)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
1.00	99.65	0.22	99.60	0.30
2.00	98.93	0.31	99.05	0.27
ZIC-HILIC-3 column				
NAL Taken(µg/mL)	Rec (%)	RSD (%)	Rec. (%)	RSD (%)
1.00	99.58	0.41	99.66	0.53
2.00	99.98	0.31	100.02	0.53
ENX Taken(µg/mL)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
1.00	99.91	0.31	99.83	0.43
2.00	99.22	0.43	99.20	0.52

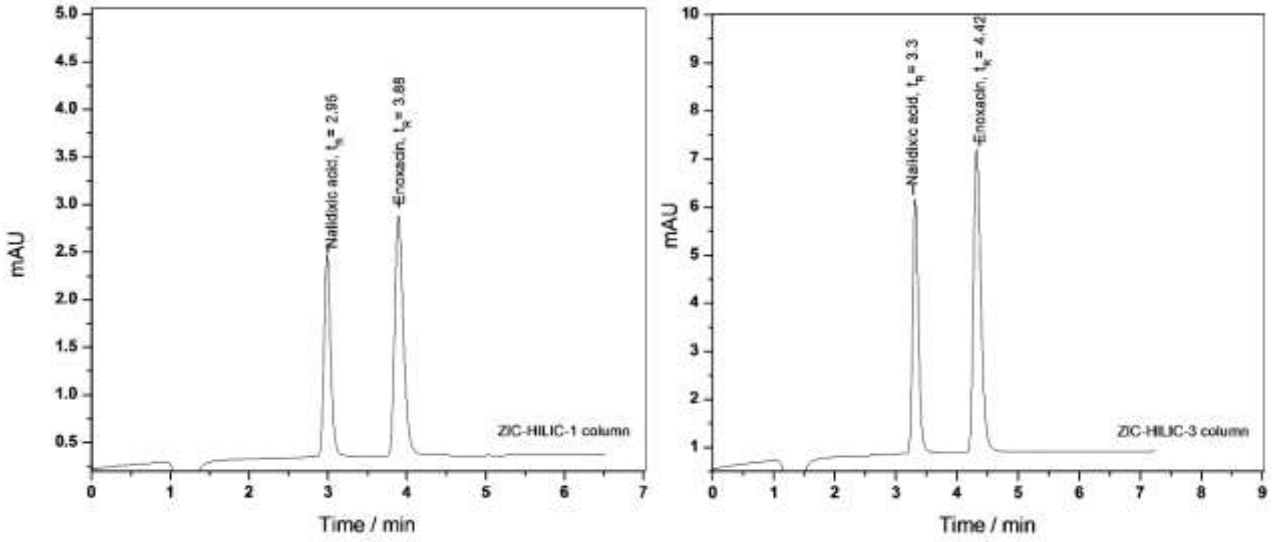

Figure 5. Chromatograms of nalidixic acid and enoxacin utilizing separation columns.

3.5. Method validation

The validation of the developed approach followed the ICH guidelines[36]. Linearity was achieved within the ranges of 0.1–6 and 0.1–12 µg mL^{-1}, with correlation coefficients of 0.9996-0.9997 and 0.9990-0.9998 for ANL and ENX, respectively (Table 1). The accuracy of the developed method was verified and evaluated by computing the percentage recoveries (%Rec) for each analyte at two distinct concentrations. Repeatability (intra-day) and intermediate precision (inter-days) were evaluated on the same day and across six distinct days. The obtained values of RSD% did not exceed 0.61%, as indicated in Table 2, confirming acceptable repeatability and intermediate precision (Table 1).

Table 2. Proposed method accuracy and precision

	Columns			
	ZIC-HILIC-1	ZIC-HILIC-3	ZIC-HILIC-1	ZIC-HILIC-3
Parameter	Nalidixic acid		Enoxacin	
Concentration range	0.1-6	0.1-6	0.1-12	0.1-12
R^2	0.9996	0.9997	0.9990	0.9998
LOD	0.070	0.04	0.080	0.060
LOQ	0.212	0.121	0.242	0.181

Table 3. The proposed technique for the quantification of NAL and ENX in medication formulas.

Brand name	Started conc. (µg/mL)	%Rec.	%RSD n=6	%Rec.	%RSD n=6
		ZIC-HILIC-1 Column		ZIC-HILIC-3 Column	
Nalidixic acid					
Nalid	1.5	100.45	0.51	100.59	0.41
NALIDIXIC	1.5	99.11	0.43	99.19	0.36
Nalidixic acid	1.5	98.32	0.66	98.28	0.73
Enoxacin					
Enoxabid	2.5	99.80	0.33	100.66	0.35
Enoxazan	2.5	98.86	0.91	98.89	0.96
Enoxacin	2.5	100.31	0.75	100.23	0.70

3.6. Quantification of ENX and NAL in medication

NAL and ENX were measured in medicinal formulations using the established chromatographic method. Table 3 lists the statistical findings obtained by looking at commercially available samples. The results (Table 4) did not exhibit any significant difference according to the Student's t-test or F-test, indicating that the suggested method can be effectively utilized to determine NAL and ENX in pharmaceutical formulations. Statistical analysis included the results of the variance ratio F-test and 95% confidence t-test (Table 4).

Table 4. Comparing the proposed method with the official method for determining NAL and ENX through t- and F-statistical tests.

Applications	ZIC-HILIC-1 Method*	ZIC-HILIC-3 Method**	USP Method	t-Test (theor.)	F-Test (theor.)
Nalidixic acid					
Nalid	100.45	100.59	99.95	0.5060* (2.7764)	5.0235* (19.000)
NALIDIXIC	99.11	99.19	100.17	0.5803** (2.7764)	5.8666** (19.000)
Nalidixic acid	98.32	98.28	99.25		
Enoxacin					
Enoxabid	99.80	100.66	100.78	0.4558* (2.7764)	1.5364* (19.000)
Enoxazan	98.86	98.89	99.66	0.7904** (2.7764)	2.4202** (19.000)
Enoxacin	100.31	100.23	99.88		

These findings are compared with those generated using the United States Pharmacopoeia technique [37] to ascertain the knowledge and effectiveness of the HILIC technique. The accuracy of the nalidixic acid and enoxacin determination in pharmaceutical forms is equal to both methods since the computed values of the t-test and F-test did not exceed the theoretical values.

4. Conclusions

This study detailed the retention behavior of NAL and ENX on two lab-made columns under HILIC conditions. The eluent strength, pH, and buffer concentration were systematically varied to evaluate their influences on the retention of the compounds. It was observed that the retention of the compounds on both columns was mainly controlled by hydrophilicity and electrostatic interaction. Different retention times were observed for NAL and ENX on both HILIC columns, which was attributed to variations in the geometric arrangement of the two phases. Specifically, the longer chain length between charged groups in the ZIC-HILIC-3 column led to an increase in retention time for both drugs. The ZIC-HILIC-3 column showed greater sensitivity, evidenced by lower LOD and LOQ, compared to the ZIC-HILIC-1 column for the chosen drugs.

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Conflicts of Interest: There is no conflict of interest to declare.

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References

[1] Armstrong, G.L.; Conn, L.A.; Pinner, R.W.; "Trends in infectious disease mortality in the United States during the 20th century" .JAMA, 281(1): 61–66, 1999.

[2] Naeem, A.; Badshah, S.L.; Muska, M.; Ahmad, N.; Khan, K.; "The current case of quinolones: synthetic approaches and antibacterial activity" .Molecules, 21(4): 268, 2016.

[3] Emami, S.; Shafiee, A.; Foroumadi, A.; "Structural features of new quinolones and relationship to antibacterial activity against Gram-positive bacteria". Mini Rev. Med. Chem., 6(4): 375–386, 2006.

[4] Bennett, J.E.; Dolin, R.; Blaser, M.J. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 9th ed.; Elsevier Health Sciences: Philadelphia, USA, 2019.

[5] Gatto, B.; Capranico, G.; Palumbo, M.; "Drugs acting on DNA topoisomerases: recent advances and future perspectives." Curr. Pharm. Des. , 5(3): 195–215, 1999.
[6] Hooper, D.C.; "Mode of action of fluoroquinolones". Drugs, 58: 6–10, 1999.
[7] Spencer, A.C.; Panda, S.S.; "DNA Gyrase as a Target for Quinolones". Biomedicines, 11(2): 371, 2023.
[8] Drlica, K.; Zhao, X.; "DNA gyrase, topoisomerase IV, and the 4-quinolones" .Microbiol. Mol. Biol. Rev., 61(3): 377–392, 1997.
[9] Wood, M.; "Tissue penetration and clinical efficacy of enoxacin in respiratory tract infections" .Clin. Pharmacokinet., 16(Suppl 1): 38–45, 1989.
[10] Jałbrzykowska, K.; Chrzanowska, A.; Roszkowski, P.; Struga, M.; "The New Face of a Well-Known Antibiotic: A Review of the Anticancer Activity of Enoxacin and Its Derivatives" .Cancers, 14(13): 3056, 2022.
[11] Eskandarian, H.; Sheikhian, L.; "Multi-walled carbon nanotube sorbent for extraction, preconcentration, and HPLC/UV determination of Nalidixic acid in human blood serum and tablet samples" . J. Iran. Chem. Soc., 18: 1657–1665, 2021.
[12] Toujani, E.; Belgaied, W.; Toujani, S.; "Simultaneous Quantitative Determination of Eight Fluoroquinolones by High-Performance Liquid Chromatography with UV Detection using a C18 Monolithic Column". Methods & Objects Chem. Anal., 18(2),101-108, 2023.
[13] Kokoszka, K.; Kobus, A.; Bajkacz, S.; "Optimization of a method for extraction and determination of residues of selected antimicrobials in soil and plant samples using HPLC-UV-MS/MS" .Int. J. Environ. Res. Public Health, 18(3): 1159, 2021.
[14] Samanidou, V.; Demetriou, C.; Papadoyannis, I.; "Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC" . Anal. Bioanal. Chem., 375(5): 623–629, 2003.
[15] Espinosa-Mansilla, A.; De La Peña, A.M.; Gómez, D.G.; Salinas, F.; "HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric detection and multiemission scanning: application to urine and serum" .J. Chromatogr. B, 822(1-2): 185–193, 2005.
[16] Daneshfar, A.; Lotfi, H.; Khezeli, T.; "Determination of nalidixic acid using dispersive liquid-liquid microextraction followed by HPLC-UV". Acta Chromatogr., 23(4): 567–577, 2011.
[17] Al-Phalahy, B.A.; Mohammed, M.J.; "Exploration of the separation mechanism of flurbiprofen and nimesulide utilizing RP-HPLC". Baghdad Sci. J.,22(4):10-21, 2024.
[18] Guo, Y.; Shah, R.; "Detailed insights into the retention mechanism of caffeine metabolites on the amide stationary phase in hydrophilic interaction chromatography". J. Chromatogr. *A*, 1463: 121–127, 2016.
[19] Fan, F.; Wang, L.; Li, Y.; Wang, X.; Lu, X.; Guo, Y.; "A novel process for the preparation of Cys-Si-NIPAM as a stationary phase of hydrophilic interaction liquid chromatography". Talanta, 218: 121154, 2020.
[20] Guo, Y.; "A survey of polar stationary phases for hydrophilic interaction chromatography and recent progress in understanding retention and selectivity." Biomed. Chromatogr., 36(4): e5332, 2022.
[21] Wang, Y.; Li, X.; Xu, X.; Wang, S.; Zhang, X.; Jiang, S.; "High efficiency and simple preparation of polyacrylamide coated silica stationary phase for hydrophilic interaction liquid chromatography". J. Chromatogr. A, 1605: 360357, 2019.
[22] Hou, Y.; Zhang, F.; Liu, X.; Ren, Y.; Yang, B. "A positively charged porous graphitic carbon stationary phase for hydrophilic interaction liquid chromatography" ,Talanta, 164: 159–163, 2017.
[23] Guo, Y.; Baran, D.; "Hydrophilic Partitioning or Surface Adsorption? A Quantitative Assessment of Retention Mechanisms for Hydrophilic Interaction Chromatography (HILIC)". Molecules, 28(18): 6459, 2023.
[24] Taylor, M.R.; Kawakami, J.; McCalley, D.V.; "Managing sample introduction problems in hydrophilic interaction liquid chromatography" .J. Chromatogr. A, 1700: 464006, 2023.
[25] Guo, Y.; Gaiki, S.; "Retention behavior of small polar compounds on polar stationary phases in hydrophilic interaction chromatography". J. Chromatogr. A, 1074(1-2): 71–80, 2005.
[26] Alpert, A.J.; "Electrostatic repulsion hydrophilic interaction chromatography for isocratic separation of charged solutes and selective isolation of phosphopeptides". Anal. Chem., 80(1): 62–76, 2008.
[27] Greco, G.; Grosse, S.; Letzel, T.; "Study of the retention behavior in zwitterionic hydrophilic interaction chromatography of isomeric

hydroxy- and aminobenzoic acids". J. Chromatogr. A, 1235: 60–67, 2012.
[28] Bicker, W.; Wu, J.; Lämmerhofer, M.; Lindner, W.; "Hydrophilic interaction chromatography in nonaqueous elution mode for separation of hydrophilic analytes on silica-based packings with noncharged polar bondings". J. Sep. Sci., 31(16-17): 2971–2987, 2008.
[29] Rasheed, A.S.; Mohammed, M.J.; "Evaluating the Selectivity of Novel ZIC-HILIC Columns and an Application for Determining Antihistaminic Drugs in Pharmaceutical Preparations". Baghdad Sci. J., 22(8):2517-2526, 2025.
[30] Mohammed, M.J.; Rasheed, A.S.; Qasim, B.; "Retention Behavior of Pantoprazole and Rabeprazole on Zwitterionic Stationary Phases in Hydrophilic Interaction Chromatography." In: AIP Conference Proceedings, Proceedings of the Conference, Cincinnati, Ohio, USA, 2025; Link, J.; Raad, H.; McGrath, S.; Al-Jadir, T.; Al-Sharif, Z. T.; TA, M. M.; Jassim, M., Eds.; Publisher: AIP Publishing, Melville, NY, USA; 2025.
[31] Qiu, H.; Loukotková, L.; Sun, P.; Tesařová, E.; Bosáková, Z.; Armstrong, D.W. "Cyclofructan 6 based stationary phases for hydrophilic interaction liquid chromatography". J. Chromatogr. A, 1218(2): 270–279, 2011.
[32] Guo, Y.; Gaiki, S. "Retention and selectivity of stationary phases for hydrophilic interaction chromatography" .J. Chromatogr. *A*, 1218(35): 5920–5938, 2011.
[33] Kawachi, Y.; Ikegami, T.; Takubo, H.; Ikegami, Y.; Miyamoto, M.; Tanaka, N. "Chromatographic characterization of hydrophilic interaction liquid chromatography stationary phases: Hydrophilicity, charge effects, structural selectivity, and separation efficiency" .J. Chromatogr. A, 1218(35): 5903–5919, 2011.
[34] Gilar, M.; Jaworski, A. "Retention behavior of peptides in hydrophilic-interaction chromatography". J. Chromatogr. A, 1218(49): 8890–8896, 2011.
[35] Rasheed, A.S.; Al-Phalahy, B.A.; Seubert, A. "Studies on behaviors of interactions between new polymer-based ZIC-HILIC stationary phases and carboxylic acids". J. Chromatogr. Sci., 55(1): 52–59, 2017.
[36] Walfish, S. "Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods". BioPharm Int., 19(12): 1–6, 2006.
[37] Heyward, J.; Padula, W.; Tierce, J. C.; Alexander, G. C. "The value of US pharmacopeial standards: a review of the literature". J. Pharm. Sci., 107(10): 2611–2617, 2018.